Cell Adherence and Its Effect on Collagen Expression in Immortalized Rat Chondrocytes (IRC) in Culture

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ABSTRACT

Chondrocytes are cells found in cartilage, and are responsible for the production and secretion of all of the molecules of the extracellular matrix (EM). Chondrocytes do not survive in the laboratory unless they have been modified in a way that “immortalizes” them. Immortalized chondrocytes serve as a valuable model system in which biomedical research can be carried out without relying on live animal models. Here, we present information on the characterization of the immortalized rat chondrocyte cell line with respect to extracellular matrix production, proliferation rates, optimal culture conditions, and the effect of cell adherence on collagen expression.

BACKGROUND

- Chondrocytes are important cells of cartilage that express most of the extracellular matrix molecules such as large proteoglycan molecules and collagen proteins including II, IX, and XI.
- 100% of collagen components of the extracellular matrix synthesized by differentiated chondrocytes is collagen II encoded by col2a1.
- Immortalization of chondrocytes increases proliferative capacity with retention of chondrocyte phenotype but decreased col2 expression and instead increased collagen XI expression.
- Since IRC cells have a chondrocyte phenotype, they are useful to study the expression of chondrocyte-specific matrix genes, and to examine the effects of environmental factors such as cytokines or growth factors on protein expression.
- In osteoarthritis for example, chondrocytes lose their ability to express collagen II and undergo apoptosis which leads to cartilage loss.
- The goal of my project is to test the effect of IRC adherence or its attachment to the flask on protein expression.
- My hypothesis is that expanded fibroblast-like IRC cells attached to the flask are still capable of expressing collagen II just like round shaped suspended cells.

RESULTS

Immortalized rat chondrocytes were cultured in an incubator in 5% CO2 at 37°C. Cells were grown using cell culture flasks. Four flasks were maintained for over four weeks. Four flasks were cultured using two different media. The experiment included cells that were attached to the flask and cells that were suspended. F12/DMEM was used to treat two flasks; one attached (Fig. 3A) and the other suspended (Fig. 3B). These flasks contained fetal bovine serum (FBS) and thus were nutrient rich and showed fast proliferation. A second medium, OPTI-MEM I was used to treat another set of flasks; one attached (Fig. 3C) and another suspended (Fig. 4D). These cells were treated with ascorbic acid to enhance protein expression.

Collagen was isolated from all flasks and treated with ammonium sulfate and left overnight. AccuSpin High Micro 17R was used to spin the media and Mammalian Protein Extraction Reagent (M-PER) was used to extract the proteins. 5% Sodium Dodecyl Sulfate- PolyAcrylamide Gel Electrophoresis was done to test for proteins expressed. Lane 1 was loaded with attached IRC in OPTI-MEM I. Lane 2+3 were both loaded with attached IRC in F12/DMEM. Lane 4 was loaded with suspended IRC in F12/DMEM and lane 5 was loaded with suspended IRC in OPTI-MEM I. Lanes 1, 4 and 5 do not show any bands in sample.

DISCUSSION and CONCLUSION

- Immortalized rat chondrocytes change morphology upon attachment showing expanded fibroblast-like shapes.
- Clusters are formed, which may be the result of adhering proteins expressed by cells.
- Suspended cells did not show any change in morphology.
- SDS-PAGE analysis showed some expression of collagen.
- Further studies could be done using immunocytochemistry to study the specific proteins expressed by these cells.

BACKGROUND

Mesenchymal stem cells differentiate into chondrocytes forming the initial cartilage. Ossification occurs at the initial cartilage longitudinally towards the epiphysis. Any irregularities in the process can affect bone formation.

Figure 1. Endochondral Bone Formation. Mesenchymal stem cells differentiate into chondrocytes forming the initial cartilage. Ossification occurs at the initial cartilage longitudinally towards the epiphysis. Any irregularities in the process can affect bone formation.

Figure 2. Enzymatic Bone Formation. Mesenchymal stem cells differentiate into chondrocytes forming the initial cartilage. Ossification occurs at the initial cartilage longitudinally towards the epiphysis. Any irregularities in the process can affect bone formation.

Figure 3. Immortalized rat chondrocytes were cultured using different media and maintained in an incubator in 5% CO2 at 37°C. A) IRC cells were grown attached and treated with F12/DMEM (nutrient rich). B) SimpRelx, no clusters of more than two cells were observed. C) IRC cultured attached, treated with OPTI-MEM I. D) IRC cultured attached, treated with OPTI-MEM I. E) Cells suspended in OPTI-MEM I, and treated with ascorbic acid did not show any change in morphology and remained round shaped.

Figure 4. A 5% SDS-PAGE was done to test for proteins expressed. Lane 1 was loaded with attached IRC in OPTI-MEM I. Lane 2+3 were both loaded with attached IRC in F12/DMEM. Lane 4 was loaded with suspended IRC in F12/DMEM and lane 5 was loaded with suspended IRC in OPTI-MEM I. Bands were observed at around 211,000 Daltons (2) indicating collagen expression by attached IRC in F12/DMEM. Lane 1, 4 and 5 do not show any obvious bands, which might indicate insufficient protein concentration in sample.